

RESEARCH ARTICLE

INVESTIGATING THE PRESENCE OF MYCOBACTERIA IN THE WATER PIPES CONSUMED IN CAFES AND TEA GARDENS

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ABSTRACT: Objectives; It's aimed to investigate the presence of Mycobacteria in the water pipes consumed in cafes and tea gardens in Hatay province. **Methods;** After determining the cafes and tea gardens which water pipes are consumed in, 250 samples were taken from water pipes. After samples were decontaminated and homogenised, they were stained with the method of Erlich Ziehl Neelsen (EZN) and were examined for acid-fast bacilli (ARB). For cultures of the samples, Lowenstein Jensen (L-J) media (BioMerieux, France) and BacT-ALERT MP (BioMerieux, France) were used. The nitrat reduction, niacin accumulation and catalase tests were made for identification of the strains isolated. **Results;** In our study, in 9 (3,6%) of 250 samples ARB were detected. Nontuberculous mycobacteria (NTM) were isolated from 27 (10,8%) samples by L-J media and from 75 (30%) samples by automated system. There was growth in both L-J as well as automated system in 24 (9,6%) samples. Thereby while isolating NTM in 78 (31,2%) of 250 samples, in none of them M. tuberculosis complex was isolated. **Conclusions;** It was thought that NTM strains might be in the tap water added into the water pipes or might be contaminated by infected users. Water pipe consumption found objectionable because of isolation of NTM in our study as NTM strains increasingly cause illness in recent years besides the many agents of infectious diseases especially M. tuberculosis.

Key words: Water pipe, *Mycobacterium tuberculosis*, nontuberculous mycobacteria

INTRODUCTION:

Tuberculosis is a contagious disease, and a significant public health problem all around the world. Infection occurs by the inhalation of droplets¹. Approximately one-third of the world's population is infected with latent tuberculosis¹. It has been remarked that

infectious diseases, e.g. tuberculosis, can also be transmitted by water pipes². Negative effects of smoking on human health are well-known in our day. However, although water pipe is based on tobacco use, it is different than cigarette in terms of its thermal and physical characteristics².

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Water pipe, or hookah, is generally used for smoking tobacco that is heated by burning embers or coal. Smoke is drawn via a rubber hose with a mouthpiece fitted to the hose's end from a water-filled (or sometimes mixed with other liquids such as wine) vase (Figure 1).



Figure 1. Water pipe

In order to remove the rigidity from the hookah tobacco, it is softened by getting blended with certain flavors such as sugary and fruity ones (orange, apple, grape, chocolate, or mint)³. The number of the organizations selling aromatized tobacco mixtures and hookahs has been increased along with the increase in hookah use. Hookah and cigarettes have gained popularity especially among youngsters living in the cities and university students³. Tobacco sales have been put into legal regulations due to rising demands. Yet, the cafes which consume water pipe have stayed out of such regulations. It is reported that the hookah use is common among adults of 18-24 age group in USA. Nevertheless, it has also been indicated that there is considerable amount of users who are secondary school and high school students as well⁴.

Hookah use is capable of arousing tendency and desire towards the use of other tobacco products. Many of the hookah users think that hookah is less harmful than cigarettes⁴. It is stated that the health

problems smokers may suffer from are also likely to be observed in hookah users. American Lung Association maintains its works in pursuit of protecting the people who do not use water pipes and are non-smokers from the harmful effects of passive smoking. Laws and regulations concerning water pipe use in public areas differ among countries. In many of them, hookah cafes are exempt from smoke-free air zone laws. Strict policies restraining the water pipe use must be carried out in order to stop this new trend, that threatening public health and is spread especially among youth. World Health Organization (WHO) gives warning about the serious risk of contamination of diseases through sharing mouthpieces of a water pipe, such as tuberculosis and hepatitis including contagious diseases⁵.

The increase, particularly in recent years, in the use of water pipes among adolescents and young adults introduces a new arena in the world in terms of tobacco control^{5,6}. Adolescents and young adults are inclined to water pipe use since they have the tendency of trying new experiences. In USA, youngsters is the group that consumes most cigarettes than all other age groups⁷.

The purpose of this study is to investigate the presence of *Mycobacteria* in the water pipes consumed in cafes and tea gardens in Hatay province.

METHODS:

Between April-October 2014, four districts in Hatay province, two of them being center and two of them being off-center, were chosen as clusters. It was aimed to reach all places within clusters with licenses to provide or sell water pipes. Samples were taken from 30 tea gardens and cafes, that is 250 water pipes in total. Districts of Hatay are shown in Figure 2. From which districts samples were taken, the number of the cafes and tea gardens samples were taken, and the number of samples is shown in Table 1.

Table 1. The districts and the number of cafes or tea garden which the samples were taken from

The Districts	The number of cafe or tea garden (N)	The number of the samples (N)
Antakya (centre)	15	125
Defne	5	56
Samandag	5	36
Arsuz	5	33
Total	30	250



Figure 2. Districts of Hatay

50 ml water extracted from the water base of water pipes were put into falcon tubes. By means of taking wipe sample from the mouth of the water pipe tube using sterile swab, and immersing it in the water in falcon tube, materials on the swab were allowed to pass to the water. The sections of the water pipe from which samples were taken are shown in Figure 3.



Figure 3. Sampling from water pipe

At first, samples were decontaminated and homogenized with NaOH Modified Petroff's Method by using 4% NaOH⁸. Treated specimens

in the falcon tube were injected to 0,5 ml L-J broth medium and 0,5 ml BacT-Alert MP broth medium. L-J broth mediums in semi-upright position and the medium facing upward were inserted into incubator, and were incubated at 37 °C until the end of 8th week. They were checked once a week. BacT-Alert MP bottles were loaded into the BacT-Alert 3D automatized system. When the device alerted, bottles were drawn out of the device and preparation was prepared to be stained via EZN method, and incubated at 37 °C by making passage to two L-J media. Catalase, niacin accumulation, and nitrate reduction tests were applied to determine whether they were *M. tuberculosis* complex or NTM. *M. tuberculosis* ATTC 25177 and *M. intracellulare* ATTC 13950 were used as control strains.

RESULTS:

After evaluating EZN-stained slides prepared out of 250 samples gathered from the water pipes consumed in cafes and tea gardens in Hatay province, ARB was detected in 9 (3,6%) of them whereas in 241 (96,4%) of them ARB was not detected. Examining the presence of ARB based on districts, ARB was found in 2 (0,8%) of the samples taken from Antakya, and 7 (2,8%) of the samples taken from Samandag. In the districts Defne and Arsuz, no ARB was found. There was no difference based on districts determined in ARB results ($p>0,05$).

Culture results were evaluated based on the growth presence in L-J and BacT-Alert MP media. NTM was isolated in 78 (31,2%) specimens. MTC was not isolated in any of the specimens. As to the analysis of the culture results according to the district-based distribution, NTM was isolated in 51 (40,8%) specimens in Antakya, 13 (23,2%) specimens in Defne, and 14 (38,9%) specimens in Samandag. No growth was observed in 33 (100%) specimens obtained from Arsuz. The number NTM isolated in Antakya and Samandag was found to be higher than the other districts

($p=0,001$). Culture results based on districts are shown in Table 2.

Table 2. The culture results according to the districts

Results of cultures	Districts				Total n (%)
	Antakya (Centre) n (%)	Defne n (%)	Samandag n (%)	Arsuz n (%)	
Negative	74 (59,2)	43 (76,8)	22 (61,1)	33 (100)	172 (68,8)
Nontuberculous Mycobacteria	51 (40,8)	13 (23,2)	14 (38,9)	0 (0)	78 (31,2)
Total	125 (100)	56 (100)	36 (100)	33 (100)	250 (100)

Table 3. The comparison between the presence of ARB and culture results

ARB	Culture results		Total N (%)	P
	No growth N (%)	Nontuberculous Mycobacteria N (%)		
ARB Negative	171 (70,9)	70 (29,1)	241 (100)	<0,001
ARB Positive	1 (11,1)	8 (88,9)	9 (100)	
Total	172 (68,8)	78 (31,2)	250 (100)	

NTM in L-J was isolated in 22 (9,1%) of 241 ARB negative samples, whereas it was isolated in 5 (55,6%) of 9 ARB positive samples ($p=0,001$). No growth was detected in L-J in 219 (90,9%) ARB negative samples, and 4 (44,4%) ARB positive samples.

In 241 ARB negative samples, NTM was isolated with automated system in 67 (27,8%) samples,

while no growth was detected in 174 (72,2%) samples. As to 9 ARB positive samples, NTM was isolated with automated system in 8 (88,9%) of them, and no growth was detected in 1 (11,1%) of them. Growth rate was found to be higher in ARB positive ones ($p<0,001$).

Comparing the ARB presence and culture results, NTM was isolated in 8 (88,9%) of 9 ARB positive samples, and in 70 (29,1%) of 241 ARB negative samples. NTM isolation rate was found to be higher in ARB positive samples ($p<0,001$) (Table 3).

NTM was isolated in L-J in 3 (1,7%) samples with no growth in automated system; in 172 (98,3%) no growth occurred in L-J. NTM was also isolated in L-J in 24 (32%) of 75 samples in which NTM was isolated in automated system. However, it was appointed that no growth occurred in L-J in 51 (68%) of them. NTM isolation rate in L-J of the samples in which NTM was isolated in automated system (32%) was observed to be higher than the rate of NTM isolation in L-J with no growth found in automated system (1,7%) ($p<0,001$).

DISCUSSION:

Water pipe is considerably common especially in the countries in Middle East and Africa. It was stated by several research conducted on university students that more than 30% of the people have used water pipe at least once in their lives⁹. Amrock et al.¹⁰ conducted a research on 18.000 American adolescents representing 27 million adolescents belonging to all nations. They reported that 2 million adolescents had been consuming water pipes until then, and 720.000 of them were inclined to use¹⁰. Another study made in USA indicated that the prevalence of water pipe use was high in 18-24 age range¹¹. Salloum et al.¹¹ obtained the result from their study that 12,4% of university graduates and 12% of family members with high annual income were using water pipe. By the research of Palamar et al.¹² conducted in the high schools in America, the prevalence of

water pipe use was found to be 18%. It was remarked that water pipe use showed variety according to demographic, socioeconomic, gender-based, racial, religious, and weekly pocket money-based differences. However, no substantial relationship between hookah use and age, family structure, and education status was found. Similar to other research, it was stated that women were using water pipes less than men, and white population were using water pipes more than colored population. It was found that the purchase of cigarettes, cannabis, alcohol or other illegal substance of those with higher pocket money was related with more frequent use of water pipes. This study determined that the students having highly educated parents featured higher possibility of using water pipes, contrary to cigarettes¹².

Water pipes can lead to the contamination of infections such as HIV/AIDS, tuberculosis, herpes, and hepatitis, because of the recurrent use of the same water pipe by many different persons and the use of the same mouthpiece several times¹². It was stated by the study of Alvur et al.¹³ that 7,89% of university students believed that using the same mouthpiece would not lead to the spread of infectious diseases.

Water pipe users assume that water pipe is less harmful than cigarettes. Research indicated that 30,6% of the students were considering smoking more harmful to human health than water pipe use, and 13,6% were thinking that water pipes are healthier due to its tobacco being blended with fruits and flavorings¹⁴. Same research also shows that the half of the participants believe that water pipe use is not as addictive as smoking. Alvur et al.¹³ asserted that 16,25% of the university students reflected that fruity and flavored water pipes were addictive, while 21,99% gave the opinion that water pipes were not addictive at all. Nevertheless, it is a known fact that through using water pipes one inhales 100 times more smoke than cigarettes. Still, young people who think water pipes contaminate diseases are greater in

number than those who think the otherwise and those who have no ideas about this issue¹³. It was determined by the study conducted among the students of the universities in Turkey that the rate of water pipe use was 29,3%, and the rate of smoking cigarettes was 18,6%¹⁴. Özcebe et al.¹⁴ asserted that 24,5% of senior students and 18,9% of first-year students were consuming water pipes. No research providing the prevalence of water pipe use in Hatay has been reached.

It is assumed that the water pipe use in our country is particularly common among young people under the age of 21. Furthermore, 62,6% of the youngsters participated in the study conducted by Şahin and Çınar¹⁵ stated that they did not have any opinion whether water pipes contaminated diseases or not.

It is forbidden in our country to sell or provide hookahs to those who are younger than 18. Water pipe consumption is not allowed in closed areas, places close to educational institutions, and workplaces without the hookah provision/presentation permit. It is required that health-related and legal warning articles explaining the risks of tobacco use must be present at the places where water pipes are consumed. However, it is a possible case that water pipe consumption not only takes place in the cafes with water pipe supply permit, but also in the cafes without such permit. Samples were not taken from these kinds of cafes without water pipe supply permit, as samples were gathered from the cafes with permit while they were being inspected. *M. tuberculosis* was not isolated in the cafes from where samples were taken.

No research made in our country that is related with water pipe use and tuberculosis has been attained. It was observed by the study of Munckhof et al.² conducted in Australia that tuberculin skin test was significant in 29 (64%) of 49 people who shared their water pipes with the ones having active tuberculosis in order to smoke marijuana. The risk of tuberculosis contamination

was calculated to be 2,2 times higher for those who share their water pipes.

NTMs are important causes of mortality in developing countries. Identification NTMs is crucial. Patients infected with *M. tuberculosis* manifest a completely different situation from the patients infected with NTM. There are various methods of isolation, detection, and determination of differences, which are used to revealed those differences between two^{16,17}. It is known that NTM isolates and the prevalence of the disease have been increased worldwide¹⁸. NTM species isolated from humans are also isolated from soil and natural water sources¹⁹.

In a study made in Asia, it was observed that 31% of the patients were clinically compatible with NTM disease. It was also remarked that MAC was the major reason behind NTM infection²⁰. Its prevalence in the world is in direction as well. It was also determined that rapidly growing mycobacteria were one of the primary reasons behind NTM disease too. It was observed during a study conducted in Holland that rapidly growing mycobacteria caused only 3% of NTM infections; this rate was measured to be 5% in USA. On the other hand, in general, it was observed that rapidly growing mycobacteria caused 14% of NTM infections; but this rate of infection rose to 30% in India, Taiwan, and South Korea²¹.

NTM infection prevalence has increased in South Korea, Canada, Denmark, Australia, USA, and Holland²⁰. According to International Union Against Tuberculosis and Lung Diseases' report of 2010, *M. fortuitum* appeared to be the most frequently

observed species, which was seen in Turkey (33,9%), Czech Republic (17,5%), Portugal (16,5%), and in other European countries²².

Shao et al.²³ investigated the geographical distribution and epidemiology of NTM species obtained from sputum samples in eastern China. At the first stage, they identified 60 of 1779

positive cultures as NTM. The number of NTM achieved from this study made in the eastern part of China was found to be lower in comparison to Shanghai, and very much lower than Europe²³. *M. intracellulare* was identified as the dominant species in the eastern part of China. The reason for this result may especially be its spread to the environment through drinking water²⁴.

Wu et al.²⁵ investigated the increase of NTM species isolated in Shanghai-China. 24763 tuberculosis cases were reported in Shanghai between those dates. The fact that 650 of them were NTM was stated; and it was determined that 5,9% increase occurred in NTM species from 2008 to 2012. Following the most commonly isolated *M. kansasii* species, *M. intracellulare*, *M. chelonae/abscessus*, *M. fortuitum*, and *M. avium* species were detected. Increasing HIV infection and immunodeficiency of the host were remarked to be connected with the increase in NTM species isolated from cultures since the past years. However, the prevalence of HIV infection is relatively low in Shanghai²⁶. Prevalence of mycobacteria varies substantially by geographical regions. Although the prevalence of NTM species are high in Asia, MAC was found to be the most isolated species; and *M. intracellulare* was the most isolated species in China²⁷.

Whiley et al.²⁸ investigated the presence of MAC species in two water distribution networks in South Australia. Average concentrations of each organism such as season, processing plant, average water temperature, and chlorine or monochloramine presence affected the results. No increase happened in MAC concentrations in

chlorine presence in water from the samples collected in winter and spring. This gives to thought that MAC would increase in hot waters containing low amount of chlorine in summer. In another distribution system, it was observed that MAC concentration increased during spring and winter times²⁸. Same research was made by Wang et al. in domestic drinking water networks in the

US. *Mycobacterium* spp. was found in high concentrations²⁹. The presence of MAC opportunistic pathogens in drinking water distribution system disinfected with chlorine and chloramine was confirmed in the study of Whiley et al.²⁸.

Albayrak et al.³⁰ evaluated the NTM species that were sent to National Tuberculosis Reference Laboratory for species identification and were isolated in the same unit. They determined by their research made in Turkey that the most commonly isolated NTM species was *M. fortuitum*, and then *M. abscessus*, *M. gordonae*, *M. avium*, *M. chelonae*, *M. intracellulare*, *M. kansasii*, *M. peregrinum*, *M. scrofulaceum*, *M. szulgai*, *M. celatum*, *M. haemophilum*, *M. smegmatis*, and *M. xenopi*³⁰. *M. fortuitum* and *M. abscessus*, which are among rapidly growing NTM species, were stated to be the most frequently observed agents. Additionally, it was emphasized that the majority of the most frequently isolated *M. fortuitum* strains were detected in the samples sent from the peripheral tuberculosis laboratories³⁰.

As seen in the studies above, NTMs are now isolated in larger numbers in clinical specimens, and are clinically acknowledged. In many countries NTMs have been isolated from pulmonary and extra pulmonary specimens, and found compatible with patients' clinics. NTMs are found in water distribution systems despite disinfectants such as chlorine. In 31,2% of the samples taken in our study NTM was isolated. NTMs may be isolated as drinking water is used in water pipes, and also because they may be contaminated to water pipes' water from the ill people using the water pipes. Infections can transmit to other people from the water pipes which are used without their water being changed and cleaned, even if the mouthpiece is replaced.

CONCLUSION:

NTM was detected in 78 (31,2%) of the specimens taken from the cafes and tea gardens (representing

Hatay province) with water pipe supply permit, whereas *M. tuberculosis* was not isolated. NTMs might have been isolated due to the tap water used in water pipes, and they might have been transmitted to the water in water pipes from infected people during the consumption of water pipes. Even if the same mouthpiece is not shared, infection can occur in people because of the use of water pipe by immunosuppressive people.

Pulmonary and extrapulmonary human infections caused by various NTM has been increasing worldwide. NTMs are widely available organisms that are widely isolated from environments such as drinking water, natural water and soil. Host factors such as genetic susceptibility, immune deficiencies and structural lung disease, as well as environmental factors including humidity, altitude, and shower systems may also affect the development of NTM lung diseases.

In order to prevent the spread of *Mycobacterium* strains, at first the inside of the water pipe must be washed with detergent, its water must be renewed, and the mouthpiece must be replaced before each use. Water pipe use should be forbidden as it can not only cause infectious diseases but also lung cancer.

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